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Immunodominance in allergic T cell reactivity to Japanese cedar in different geographical cohorts

Carla Oseroff, MSc¹, John Pham, BSc¹, April Frazier, PhD¹, Denise Hinz, PhD¹, John Sidney, MSc¹, Sinu Paul, PhD¹, Jason A Greenbaum, PhD¹, Randi Vita, PhD¹, Bjoern Peters, PhD¹, Véronique Schulten, PhD¹, and Alessandro Sette, PhD¹

¹La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA

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Introduction

Japanese cedar (JC) pollen is one of the most frequently inhaled outdoor allergens in Japan and a potent inducer of seasonal allergic asthma and rhinitis. Epidemiological studies of JC pollinosis have reported a more than 2.5-fold increase in JC allergy over the last 3 decades¹. In recent years, different surveys conducted to estimate the prevalence of JC pollinosis have reported a prevalence ranging from 13 to 36% in the Japanese population, making it a major national health problem in Japan^{2, 3}. This striking increase is believed to be associated with environmental factors such as reforestation and climate change. The first studies describing JC pollinosis were published in the 1960s^{4, 5}, and disease prevalence has been increasing ever since. Retrospective studies have associated this trend with the expansive afforestation era in Japan conducted from the 1950s to 1970s. During this period, billions of Japanese cedar trees (*Cryptomeria japonica*) were planted as a future source of construction material. However, decreasing wood tariffs and imported wood diminished the need for JC as construction material resulting in massive amounts of land covered by forests made up entirely of Japanese cedar trees at an optimum age for pollen production^{6, 7}. In addition, climate change is promoting flower bud development and increasing pollen production^{7, 8}.

To date, two major allergens in Japanese cedar pollen have been described; Cry j 1 and Cry j 2^{9, 10}. Both allergens are highly dominant, eliciting IgE reactivity in > 90% of JC-allergic patients tested¹¹. In addition to Cry j 1 and 2, a number of reports have been published on other JC-derived allergenic proteins, including Cry j 3, a theumatin-like protein^{12, 13}, Cry j 4, a calcium-binding protein¹⁴, Cry j isoflavone reductase (IFR), which is related to the minor birch allergen Bet v 6 (62 % sequence identity)¹⁵, Cry j chitinase, a class IV chitinase^{16, 17},

Corresponding Author: Véronique Schulten, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037., Telephone: 858-752-6922, Fax: 858-752-6985, veronique@lji.org.

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Cry j Asp, an aspartic protease¹⁸, Cry j LTP, a lipid transfer protein¹⁹ and Cry j CPA9, a serine protease²⁰. Despite this relative abundance of allergenic proteins in Japanese cedar, only Cry j 1 and 2 are considered major allergens and as a result have been the focus of more detailed studies. The T cell response to both Cry j 1 and 2 has been thoroughly characterized in Japanese patients and several immune-dominant regions have been identified^{21–23}.

Although considerable efforts have been made to improve our understanding of the immunological processes involved in JC pollinosis, the vast majority of these studies have been performed in cohorts of JC pollen allergic, Japanese patients. However, given the degree of potential cross-reactivity of JC pollen with other tree pollens such as Italian cypress²⁴, studies in non-Japanese subjects who have IgE reactivity against JC pollen are of great interest. Furthermore, studies on differences between immune recognition and responses of non-sensitized and sensitized donors in the JC system are extremely rare. Several reports suggest that non-sensitized individuals mount an immune response distinct from those of allergic individuals and are not oblivious to antigen stimulation^{25–27}. Thus defining the features and targets of healthy individuals is important and understudied, and could provide insights for the development of safer and more efficacious immunotherapeutic approaches.

In this report, we aim to evaluate the effect of JC pollen exposure on the T cell response specificity and magnitude in allergic and non-allergic individuals. Studies measuring Japanese cedar pollen in Japan over a span of 10 years or more have reported detectable levels from November until April, with levels varying from 1–20 pollen grains/cm² per months, accumulating 500–12000 pollen grains/cm² over the entire season^{28, 29}. In contrast, Japanese cedar and other related and cross-reactive species are not indigenous in Southern California and are hence not considered a major allergen. However, grasses (e.g. *Cynodon dactylon*, *Lolium perenne*, *Phleum pratense*) and ragweed (*Ambroisa artemisiifolia*) pollen do grow in Southern California and levels ranging from 4–50 pollen grains/cm² per months can be detected throughout the respective season (levels determined by Erik and Ese Banck Clinical Research Center, San Diego, as reported by the American Academy of Allergy, Asthma and Immunology).

Here, we study the T cell response to three JC allergens in allergic and non-allergic Japanese patients, presumed to be exposed, versus Southern Californian patients who have never been to Japan to evaluate how exposure and sensitization influence the response magnitude and epitope-specificity of allergen-specific T cells, focusing the Cry j 1, 2 and IFR as a representative minor allergen.

Methods

Study population

Blood donations were collected (450 ml) from Japanese cedar-allergic and non-allergic patients. Each donor was recruited following institutional review board approval (IRB number VD-112). Written informed consent for study participation was obtained. At the time of donation, information regarding whether prospective donors had lived in Japan for at least 1 year was also collected and donors were classified as “lived in Japan” (LIJ or “not

lived in Japan” (NLJ). Serum samples were tested for JC pollen (Cry j)-specific IgE by the ImmunoCAP using natural JC pollen extract (Phadia, Upsala, Sweden), and donors were classified as JC-sensitized or not (Cry j IgE+/ Cry j IgE-) based on a positivity threshold of >0.35 kU_A/L. Based on the criteria described above the study population was divided in the following three sub-cohorts (Table 1): 1) LIJ IgE+ (n=10); 2) NLJ IgE+ (n=24) and 3) LIJ IgE- (n=20).

Polysensitization determination by ImmunoCAP

In addition to Japanese cedar, plasma samples from all donors were additionally tested by ImmunoCAP (Phadia, Upsala, Sweden) for IgE titers against a panel of 13 natural extracts made from allergenic species including tree pollen (Italian cypress (Cup s), Mountain cedar (Jun s), Red cedar (Jun v) Birch (Bet v), Oak (Que e), Olive (Ole e) and White Ash (Fra a)), grasses (Timothy (Phl p), Bermuda (Cyn d), Sweet vernal (Ant o), Kentucky blue (Poa p) and Rye (Lol p)) and weed (Ragweed (Amb a)) species.

Selection of peptides from Japanese cedar allergen sequences

Sequences of all known Cry j 1 and Cry j 2 isoforms were obtained from the WHO/IUIS Allergen Nomenclature database (www.allergen.org)³⁰. In addition, the sequence from Cry j IFR was obtained from the NCBI protein database (Accession number AAK27264.1). Sets of peptides of 16 amino acids in length, overlapping by 8 residues, were generated to cover all three allergen protein sequences. Peptides from isoforms were synthesized as well as any other peptides appearing in at least 3 (25%) isoforms. Overall, a total of 148 peptides were assembled in 15 pools of ~10 peptides each, and screened for their T cell reactivity.

Peptide synthesis

Peptides were purchased from A and A (San Diego, CA) as crude material on a small (1 mg, >70% purity) scale. Individual peptides were resuspended in DMSO at a final concentration of 40 mg/ml.

Stimulation and expansion of Japanese cedar-specific T cells

PBMCs were isolated from whole blood by density gradient centrifugation according to manufacturers' instructions (Ficoll-Hypaque, Amersham Biosciences, Uppsala, Sweden) and cryopreserved. For *in vitro* expansion of JC-specific T cells, PBMCs were thawed and stimulated with JC pollen extract (ALK-Abello A/S, Horsholm, Denmark) at 2 µg/ml. Cells were cultured in RPMI 1640 supplemented with 5% human AB serum in 24 well plates (BD Biosciences, San Diego, CA) at a density of 2×10^6 /ml and incubated at 37 °C. IL-2 (10 U/ml) was added every 3 days after initial stimulation. Cells were harvested on day 14 and screened for reactivity to peptide pools by ELISPOT.

Dual ELISPOT assays

The production of IFN γ and IL-5 from cultured PBMCs in response to antigenic stimulation was assessed by dual ELISPOT assays as described previously³¹.

Peptide pools that elicited a positive response (>100 spot forming cells/ 1×10^6 PBMC) were deconvoluted to identify the individual epitopes at day 17 using cells from the same original culture.

Statistical correlation to determine hierarchy of T cell reactivity

To compare the T cell epitope repertoire in each cohort for each of the 3 Cry j allergens, average responses to each peptide from all three cohorts were compared by Spearman's rank correlation analysis, a two-tailed, non-parametric measure of rank correlation. The R^2 value was calculated from the Spearman r value. P-values <0.05 are considered significant.

HLA typing and restriction

HLA typing for Class I (HLA-A; HLA-B; HLA-C) and Class II (HLA-DQA1; HLA-DQB1, HLA-DRB1,3,4,5; HLA-DPB1) was performed by an ASHI-accredited (American society for histocompatibility and immunogenetics) laboratory at Murdoch University (Western Australia) as previously described³². Potential HLA-epitope restriction odds ratios and relative frequencies were calculated using the RATE program³³.

Results

Higher JC pollen-specific IgE titers and different polysensitization patterns in allergic patients who have lived in Japan (LIJ IgE+) compared to allergic patients who have not (NLJ IgE+)

JC pollen-specific IgE titers in LIJ IgE+ and NLJ IgE+ patients were determined by ImmunoCAP (Figure 1A). As expected on the basis of the likely exposure history, titers were significantly higher in the LIJ IgE+ cohort (median 7.46, range 0.73–17.62 kU/L) compared to the NLJ IgE+ cohort (median 1.28, range 0.41–9.01 kU/L).

Next, we analyzed the level of polysensitization to other tree, grass and weed pollens. For this purpose, IgE titers from the LIJ IgE+ and NLJ IgE+ to a panel of 13 allergenic species were assessed (Figure 1B). The test panel was grouped into 4 categories: 1. JC-related tree pollens; 2. other tree pollens; 3. grass pollens and 4. weed pollen. Interestingly, in the LIJ IgE+ cohort, highest IgE titers were observed to Cry j (median 7.5 kU/L). Other sensitizations in this cohort were effectively limited to JC-related tree species, namely red cedar (Jun v, median titer 2.03 kU/L) and mountain cedar (Jun s, median titer 1.46 kU/L) (Figure 1B). In contrast, in the NLJ IgE+ cohort, IgE titers measured were mostly targeted towards grasses and weeds. As described above, JC pollen (Cry j) IgE titers were much lower compared to the LIJ patients (median 1.28 kU/L). Similar titers were observed for JC-related trees and other tree pollen (Figure 1B). The highest titers were observed for Kentucky blue (Poa a) and Ryegrass (Lol p) (22.4 and 18.6 kU/L, respectively). These differences in IgE titers to a panel of common pan-pollen allergens suggest fundamental differences in the exposure and origin of sensitization.

JC pollen-specific T cell responses are significantly higher in allergic patients who have lived in Japan (LIJ IgE+) compared to allergic patients who have not (NLJ IgE+)

We then determined T cell reactivity (expressed as the sum of IL-5 and IFN γ producing cells) to JC extract, and the Cry j 1, 2 and Cry j IFR allergens in all 3 cohorts (Figure 2). Panels of 16-mer peptides, overlapping by 8 residues and spanning the Cry j allergen sequences, were generated and screened in pools of ~10 for IFN γ and IL-5 produced by *in vitro* JC pollen extract expanded PBMC in ELISPOT assays. Positive pools were deconvoluted to identify individual epitopes. For each allergen, overall T cell reactivity is expressed as the sum of individual peptide responses observed in each donor (Figure 2). Responses to medium and PHA stimulation are shown in Supplemental Figure 1.

In the case of the JC pollen extract, responses were highest in the LJI IgE+ cohort, with all patients responding with a median magnitude of 3080 SFC (Figure 2A). In the NLJ IgE+ cohort extract also elicited detectable T cell reactivity in all patients tested with a median response magnitude of 1857 SFC (Figure 2A). As expected, in the non-allergic LIJ cohort (LJI IgE-), T cell response magnitudes and frequencies to JC pollen extract were lower than in the NLJ IgE+ and LJI IgE+ cohorts, eliciting positive responses in 17/20 (85%) donors with a median response of 1103 SFC (Figure 2A).

Similar to extract T cell responses, reactivity to allergen-derived peptides was also high. For Cry j 1 (Figure 2B), 90% of the LJI IgE+ cohort donors responded, with a median response across the entire cohort of 7430 SFC. In the NLJ IgE+ cohort, T cell responses were significantly lower. Responses were detected in 14/24 donors (58%) with a median magnitude of 160 SFC (Figure 2B). In the LIJ IgE- cohort, 8/20 (40%) patients responded to Cry j 1 with magnitudes ranging from 60–3411 SFC (Figure 2B).

For Cry j 2, 100% of LJI IgE+ donors responded with a median response of 5899 SFC (Figure 2C). In the NLJ IgE+ cohort, 12/24 (50%) patients responded with a median magnitude of 35 SFC. Surprisingly, 12/20 (60%) of LJI IgE- donors responded to Cry j 2 with a median magnitude of 222 SFC (Figure 2C). Finally, no T cell responses against Cry j IFR were detected in the LJI IgE+ cohort (Figure 2D). T cell responses were observed in only 5/24 (21%) of NLJ IgE+ donors with magnitudes ranging from 93 to 1383 SFC, whereas reactivity was detected in 4/20 (20%) of the LIJ IgE- donors (magnitudes ranged from 57–543 SFC) (Figure 2D).

T cell epitope recognition and immunodominance in the 3 donor cohorts

Next, we further analyzed individual T cell epitopes from the three allergens for each donor cohort. Figure 3 A–C shows the magnitude of recognition of the three Cry j allergens and Figure 3D–F shows the corresponding frequencies. Due to the differences in response magnitudes between cohorts, data in Figure 3 A–C is plotted using two different scales (the LIJ IgE+ cohort is plotted according to the scale shown on the left axis, while data from the NLJ IgE+ and LJI IgE- cohorts are plotted according to the scale shown on the right axis) to facilitate the visualization of T cell dominance.

Overall, we identified 117 T cell reactive peptides (43 from Cry j 1, 57 from Cry j 2 and 17 from Cry j IFR). To the best of our knowledge, 27 of these epitopes have never been

reported before (3 from Cry j 1, 12 from Cry j 2 and 12 from Cry j IFR; no matching entry in the Immune Epitope Database³⁴). Different epitopes were dominant within each cohort. The strongest responses were observed in the LIJ IgE+ cohort, with the most dominant 11 peptides (inducing an average response >400 SFC) accounting for 40% of the total response in that cohort. Responses in the NLJ IgE+ and LIJ IgE- cohorts were similarly dominated by a small number of peptides. In both cohorts the 6 strongest peptides, eliciting an average of 90 SFC or higher, accounted for 40% or more of the total response. Sequences, average response magnitudes and frequencies for all peptides for each allergen are summarized in Table 2.

In the T cell response of LIJ IgE+ patients to Cry j 1, the most dominant peptide with respect to response magnitude was Cry j 1₂₈₁ inducing average response of 722 SFC, recognized in 60% of LIJ IgE+ patients. The most frequently recognized peptide was Cry j 1₈₉, recognized in 70% of donors. In the case of NLJ IgE+ patients, Cry j 1₂₄₉ (average of 124 SFC) induced the most dominant response but Cry j 1₂₃₃ was most frequently recognized (positive in 26% of patients). In the LIJ IgE- cohort, Cry j 1₄₁ was mostly strongly recognized (93 SFC on average) and Cry j 1₁₂₁ and Cry j 1₁₆₉ were highest in recognition frequency (15%).

In the case of Cry j 2, the most dominant peptide in the LIJ IgE+ patients, Cry j 2₁₂₁, was shared in the LIJ IgE- cohort, inducing an average T cell response of 888 and 226 SFC, respectively. It was among the most frequently recognized peptides in both cohorts (recognized in 25% of the LIJ IgE- cohort and 70% in the LIJ IgE+ cohort). The second most dominant peptide in the LIJ IgE+ patients, Cry j 2₄₀₁, did not elicit strong responses in either of the other 2 cohorts. In the NLJ IgE+ patients, Cry j 2₉₇ elicited most prominent responses (average of 157 SFC, 17% recognition frequency) but the most dominantly recognized peptide in NLJ IgE+ patients was also Cry j 2₁₂₁, which was seen in 22% of patients.

For Cry j IFR, responses were much weaker overall and no significant overlap of T cell epitope repertoire was observed (Table 2). Detectable T cell responses against Cry j IRF peptides were only observed in NLJ IgE+ patients, with the most dominant peptide, Cry j IFR₁₂₁, triggering an average response of 26 SFC, being reactive in 17% of patients.

T cell responses from JC pollen allergic and non-allergic patients have a similar hierarchy of epitope recognition

Despite the fact that the most reactive epitopes differed in the three cohorts, several epitopes were strongly recognized in multiple cohorts (e.g. Cry j 1₁₇₇, Cry j 1₂₃₃, Cry j 2₁₂₁, Cry j 2₂₁₇ and Cry j 2₃₆₁; Table 2). To address this further, we compared the T cell epitope repertoire in each cohort for each of the 3 Cry j allergens by performing a Spearman's rank correlation analysis. Reactivity to each peptide within each cohort can be found in the Immune Epitope Database (IEDB submission ID 1000703; www.iedb.org). Analysis of T cell reactivity against Cry j 1-derived peptides revealed that the hierarchy of reactivity against the various peptides in all three cohorts correlates significantly (Figure 3A). Patterns of epitope recognition were most similar between LIJ IgE+ and NLJ IgE+ patients ($R^2=0.34$ and $p<0.001$, all values shown in Table 3). The second strongest overlap in T cell reactive regions was observed between LIJ IgE+ and IgE- patients ($R^2=0.19$ and $p=0.003$). Epitopes

recognized by NLJ IgE+ and LIJ IgE- were least similar but the correlation still reached statistical significance ($R^2 = 0.09$ and $p = 0.043$, Table 3). Similarly to Cry j 1, correlations of peptide recognition for Cry j 2 between cohorts were significant overall (Table 3), however the hierarchies of overlap were distinct from Cry j 1 (Figure 3B). The greatest overlap in epitope repertoire was observed between the LIJ IgE+ and IgE- cohorts ($R^2 = 0.4$ and $p < 0.001$), followed by the LIJ IgE+ and NLJ IgE+ cohorts ($R^2 = 0.16$ and $p = 0.001$) and lastly the NLJ IgE+ and LIJ IgE- cohorts ($R^2 = 0.14$ and $p = 0.002$).

Inferred HLA restrictions of dominant epitopes

We and others previously reported that allergen epitopes are rather heterogeneous in terms of HLA restrictions^{31, 35}. That is, while DR-restricted responses were the most prevalent, DQ and DP restrictions were also identified, with some epitopes restricted by multiple loci. Here, to identify potential HLA restrictions, and to facilitate the design and use of HLA tetramers, we used the RATE program³³ to calculate the relative frequency and significance of association between all the epitopes/regions and HLA alleles (or combinations thereof) expressed in responding donors.

A detailed account of the results of the RATE analysis is shown in Table 4, which gives the number of donors that responded (R+) or did not respond (R-) to a given peptide, and expressed (A+) or did not express the given HLA(s) (A-). For example, the Cry j 1₂₃₃ epitope has 100% of the responders express the HLA molecules DPB1*05:01, DRB5*01:01 or DRB1*04:01, while only 7/16 (44%) of the non-responders express the any of these same HLAs ($p = 0.001$). This analysis allowed inference of potential restrictions for a majority of the main epitopes (Table 4). Of the 20 cases where restrictions could be inferred, all were promiscuous, i.e. the epitope is inferred to be potentially restricted by multiple HLAs, thus confirming and extending the previous results³¹.

Discussion

In the present study, we compared patterns of immunodominance in T cell recognition to several Cry j allergens in a cohort of sensitized (IgE+) individuals that have resided in Japan for a minimum of 12 months versus sensitized individuals from Southern California who, to the best of our knowledge never resided in Japan. We find that the two cohorts have commonalities in terms of T cell immunodominance at the antigen level, and even at the level of epitope recognition. This finding was contrary to our expectations, because the two cohorts differ in many important aspects, such as ethnicity, JC-specific IgE titers, pattern of polysensitization to other allergens, and most importantly the presumed level exposure to JC pollen.

To the best of our knowledge, ours is the first side-by-side comparison of human T cell reactivity to various Cry j allergens in JC-allergic patients who have lived in Japan versus allergic patients who have not. We found that T cell reactivity correlates with the reported dominance of IgE responses against the same allergens, in that IFR IgE reactivity is observed less frequently (76%)¹⁵ compared to Cry j 1 and Cry j 2 (> 90%)¹¹. Indeed despite the reports of Kawamoto et al.¹⁵, who show IgE reactivity in a majority of individuals tested, T cell reactivity to this allergen was negligible in all 3 cohorts.

With respect to the magnitude of immunological reactivity to JC pollen, we found that the NLJ IgE+ donors exhibited much lower immune-reactivity both at the serological and T cell level. This lower reactivity may reflect low/infrequent exposure (travel to Japan, exposure to Japanese cedar plants cultivated in the USA, and/or cross-reactive species). The substantial difference in reactivity between the two cohorts is underlined by the drastic difference in the pattern of polysensitization, which indicates that NLJ IgE+ patients have much higher IgE titers to grasses and weeds, whereas LIJ IgE+ patients are mostly IgE reactive to JC-related tree pollens.

Comprehensive mapping of T cell epitopes for each allergen revealed that overall patterns of reactivity overlap significantly between both IgE+ cohorts irrespectively of their geographical location, and even IgE- control donors. These commonalities were somewhat surprising, given the many differences between the various cohorts. This finding can be reconciled in light of several reports that highlight how a significant overlap exists between different HLA class II allelic variants, and that epitopes capable of binding multiple HLAs (promiscuous epitopes) account for a significant fraction of overall T cell reactivity³¹.

Despite the significant overlap, for each allergen and each cohort, unique dominant peptides were also identified. These differences are potentially explained by differences in the frequency of different HLA class II allelic variants in the different cohorts, and also magnified by the relative small number of donors tested in each cohort.

Several studies have been conducted to identify T cell epitopes from Cry j 1 and 2, however these studies have largely been focused on JC pollen allergic individuals from Japan. As has been reported before²², Cry j-specific T cell reactivity is also detected in non-sensitized individuals, albeit at a much lower magnitude and frequency. Analyzing the T cell response to these allergens in non-sensitized individuals and patients who have JC pollen-specific IgE but have never lived in Japan allowed to define sets of epitopes that are of broad potential utility, as they would be active in different cohorts of individuals, associated with large differences in exposure and ethnicity. Our data emphasizes the value of performing studies evaluating antigens and epitopes in different geographical settings, as has been done previously in different disease settings^{36, 37}.

Here we defined 117 Cry j-derived epitopes, 27 of which have to the best of our knowledge never been reported in the literature before. The identification of dominant T cell epitopes provides a tool that can be used to study the immunological characteristics and modulatory changes of the allergic T cell response before and after therapy, using it as immunological assessment for treatment efficacy as has been done in the Timothy grass system^{38, 39}. To further facilitate antigen-specific T cell studies, we used the RATE program, which can infer restriction elements for the more dominant epitopes. This data will allow the production of tetrameric staining reagents to be used in future studies characterizing Cry j-specific T cell responses in context of allergic disease and immunotherapy.

This comprehensive characterization of T cell reactivity in Japanese and non-Japanese allergic and non-allergic individuals is of high relevance for the development of immunotherapeutic approaches. A clear understanding of similarities and differences in the

T cell response in patients with true sensitization versus co-recognition due to sensitization to a cross-reactive allergen⁴⁰ is essential to project efficacy of different therapeutic reagents in these distinct cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest

Alessandro Sette and Bjoern Peters are consultants for ALK-Abelló A/S Bøge Allé 6 DK-2970 Hørsholm, Denmark.

Abbreviations

IFR	isoflavone reductase
PBMC	Perioheral blood mononuclear cells
JC	Japanese cedar
LIJ	lived in Japan
NLJ	not lived in Japan
IFNg	interferon gamma
IL	interleukin
ASHI	American society for histocompatibility and immunogenetics

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Figure 1.

Bar graphs depicting the median of A) Japanese cedar-specific IgE titers (kU_A/L) in allergic patients who lived in Japan (LIJ) and allergic patients who did not (NLJ) and B) IgE titers to a panel of 13 pan pollen allergens in LIJ patients (upper panel) and NLJ patients (lower panel). Error bars indicate interquartile range. Statistical analysis was performed by Mann-Whitney test (non-parametric, two-tailed), ***- $p < 0.001$. LIJ cohort: $N=10$; NLJ cohort: $N=24$

Figure 2.

Bar graphs depicting median values of total allergen-specific T cell responses (sum of IL-5 and IFN γ responses to extract or individual peptides) to A) JC extract, B) Cry j 1, C) Cry j 2 and D) Cry j IFR in all 3 cohorts tested. Each symbol represents a single donor. Error bars indicate interquartile range. Statistical analysis was performed by Mann-Whitney test (non-parametric, two-tailed), ** - $p < 0.01$, *** - $p < 0.001$, **** - $p < 0.0001$. LIJ IgE+ cohort: N=10; NLJ IgE+ cohort: N=24; LIJ IgE- cohort: N=20

Figure 3.

Average epitope-specific T cell responses (A–C) (sum of IL-5 and IFN γ) and response frequency (D–F) for each individual peptide spanning A and D) Cry j 1, B and E) Cry j 2 and C and F) Cry j IFR in LIJ IgE+, NLJ IgE+ and LIJ IgE– donors. Due to the differences in response magnitudes between cohorts, data in panels A–C is plotted using two different scales. The LIJ IgE+ cohort is plotted according to the scale shown on the left axis, data from the NLJ IgE+ and LIJ IgE– cohorts are plotted according to the scale shown on the right axis). LIJ IgE+ cohort: N=10; NLJ IgE+ cohort: N=24; LIJ IgE– cohort: N=20

Table 1

A summary of epidemiological and clinical donor characteristics.

Donor	Cohort	Age	Gender	Cry j IgE (kU/L)	Time lived in Japan	Year	Location
1569	LJ IgE+	40	F	2.71	9 years	n.d.	Yamanashi, Kofu
2098	LJ IgE+	20	F	8.76	7 years	2002	Nagoya, Archi Preetune
2102	LJ IgE+	24	F	14.08	17.5 years	2009	Tokyo
2144	LJ IgE+	45	F	0.73	40 years	2015	Kobay
2188	LJ IgE+	40	F	5.45	2.5 years	2003	sasebo
2199	LJ IgE+	25	F	4.99	15 years	2005	Tokyo
2212	LJ IgE+	32	M	17.62	13 years	2015	Kanamoto, Tokyo
2220	LJ IgE+	21	M	13.42	22 years	2013	Kanagawa, Tokyo
2254	LJ IgE+	31	F	6.45	28 years	2014	Inago?, Tokyo
2258	LJ IgE+	25	F	8.46	24 years	2014	Tokyo
1005	NLJ IgE+	31	M	0.59	N/A	N/A	N/A
1006	NLJ IgE+	39	M	0.67	N/A	N/A	N/A
1014	NLJ IgE+	58	M	3.64	N/A	N/A	N/A
1020	NLJ IgE+	32	F	0.61	N/A	N/A	N/A
1174	NLJ IgE+	50	M	0.55	N/A	N/A	N/A
1175	NLJ IgE+	38	F	3.39	N/A	N/A	N/A
1191	NLJ IgE+	41	F	0.6	N/A	N/A	N/A
1198	NLJ IgE+	23	M	3.49	N/A	N/A	N/A
1363	NLJ IgE+	37	M	0.89	N/A	N/A	N/A
1381	NLJ IgE+	56	M	2.2	N/A	N/A	N/A

Donor	Cohort	Age	Gender	Cry j IgE (kU/L)	Time lived in Japan	Year	Location
1435	NLJ IgE+	36	M	0.69	N/A	N/A	N/A
1437	NLJ IgE+	34	F	1.89	N/A	N/A	N/A
1440	NLJ IgE+	25	M	0.49	N/A	N/A	N/A
1441	NLJ IgE+	43	M	0.41	N/A	N/A	N/A
1453	NLJ IgE+	20	M	0.98	N/A	N/A	N/A
1460	NLJ IgE+	30	M	1.69	N/A	N/A	N/A
1652	NLJ IgE+	56	F	1.31	N/A	N/A	N/A
1864	NLJ IgE+	34	F	9.01	N/A	N/A	N/A
2017	NLJ IgE+	21	M	1.28	N/A	N/A	N/A
2021	NLJ IgE+	18	F	1.93	N/A	N/A	N/A
2036	NLJ IgE+	31	M	1.38	N/A	N/A	N/A
2201	NLJ IgE+	20	M	0.59	N/A	N/A	N/A
2240	NLJ IgE+	27	F	1.84	N/A	N/A	N/A
1553	LJ IgE-	23	F	0	7 years	1999	n.d.
1578	LJ IgE-	56	F	0.03	n.d.	n.d.	n.d.
1591	LJ IgE-	21	F	0.28	6 years	n.d.	Hiroshima
1616	LJ IgE-	41	M	0	5 years	1996	yokasuka, Atsugi
1617	LJ IgE-	30	F	0.02	4.5 years	2014	Kyoto
2095	LJ IgE-	26	F	0	3 years	2014	Yogi
2145	LJ IgE-	52	M	0.13	3 years	2014	Kabay
2186	LJ IgE-	51	F	0.01	28 years	1993	Tokyo
2187	LJ IgE-	45	M	0	2 years	1992	Tokyo, Yokohima
2221	LJ IgE-	56	M	0	1.5 years	1982	Okanawa

Donor	Cohort	Age	Gender	Cry j IgE (kU/L)	Time lived in Japan	Year	Location
1599	LJI IgE- a1	33	M	0.05	32 years	2013	Hyogo, Tokyo
1626	LJI IgE-	23	M	0.03	5.5 years	2007	Okanawa
1632	LJI IgE-	54	M	0.07	9 years	n.d.	Hiroshima
1635	LJI IgE-	41	F	0.06	26 years	1999	Hiroshima
1694	LJI IgE-	32	F	0	N/A	n.d.	N/A
2185	LJI IgE-	31	F	0.01	5 years	1989	Okanela
2198	LJI IgE-	29	F	0	23 years	2009	Tokyo
2234	LJI IgE-	18	F	0.02	10 months	2000	Nishinomiya
2237	LJI IgE- a1	20	F	0.01	20 years	2015	Tokyo
2239	LJI IgE-	29	F	0	1 year	2013	Kyoto

Table 2

Summary of average response magnitudes and frequencies for each peptide spanning Cryj 1, Cry j 2 and Cryj IFR for each donor cohort.

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)			
			LIJ IgE+	SD	NLJ IgE+	St. Dev	LIJ IgE-	SD	LIJ IgE+	NLJ IgE+	LIJ IgE-	
1	Cry j 1	MDSPLVALLVFSFVI	30	68	3	17	0	0	20	4	0	
9	Cry j 1	LLVFSFVIGSCFSDNP	9	28	4	19	0	0	10	4	0	
17	Cry j 1	GSCFSDNP IDSCWRGD	110	349	0	0	0	0	10	0	0	
25	Cry j 1	IDSCWRGDSNWAQNRM	147	466	0	0	29	129	10	0	5	
33	Cry j 1	SNWAQNRMKLADCAVG	97	308	0	0	29	90	10	0	10	
41	Cry j 1	KLADCAVFGSSTMGG	39	122	7	35	93	404	20	4	10	
49	Cry j 1	FGSSTMGGKGGDLYTV	18	58	0	0	0	0	10	0	0	
57	Cry j 1	KGGDLYTVTNSDDDPV	64	203	8	41	0	0	10	4	0	
65	Cry j 1	TNSDDDPVNPAPGTLR	16	50	0	0	6	28	10	0	5	
73	Cry j 1	NPAPGTLRYGATDRDP	384	928	0	0	0	0	20	0	0	
81	Cry j 1	YGATDRDRPLWII FSGN	72	141	3	15	0	0	30	4	0	
89	Cry j 1	LWII FSGNMNIKLKMP	401	490	4	18	54	200	70	4	10	
97	Cry j 1	MNIKLKMPMYIAGYKT	153	322	91	444	0	0	20	4	0	
105	Cry j 1	MYIAGYKTFDGRGAQV	210	448	8	30	0	0	30	9	0	
113	Cry j 1	FDGRGAQVYI GNGGPC	218	280	8	37	54	199	60	4	10	
121	Cry j 1	YI GNGGPCVFIKRVSN	231	438	22	75	90	251	30	9	15	
129	Cry j 1	VFIKRVSNVIIHGLYL	363	495	45	146	3	13	60	17	5	
137	Cry j 1	VIIHGLYLYGCSTSVL	29	65	0	0	0	0	20	0	0	
145	Cry j 1	YGCSTSVLGNVLINES	0	0	0	0	0	0	0	0	0	
153	Cry j 1	GNVLINESFGVEPVHP	67	130	0	0	0	0	30	0	0	
161	Cry j 1	FGVEPVHPQDGDALT L	172	368	0	0	0	0	20	0	0	
169	Cry j 1	QDGDALT LRTATNIWI	54	134	0	0	63	176	30	0	15	
177	Cry j 1	RTATNIWIDHNSFSNS	543	832	121	593	53	175	50	4	10	
185	Cry j 1	DHNSFSNSDGLVDVT	82	150	15	74	0	0	40	4	0	

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)			
			LJ IgE+	SD	NLJ IgE+	St. Dev	LJ IgE-	SD	LJ IgE+	NLJ IgE+	LJ IgE-	
193	Cry j 1	SDGLVDVTLTSTGVTL	77	167	3	15	0	0	30	4	0	0
201	Cry j 1	LTSTGVTLISNNLFFNH	13	40	0	0	0	0	10	0	0	0
209	Cry j 1	SNNLFFNHKKVMLLGH	414	852	16	45	18	60	30	13	10	10
217	Cry j 1	HKVMLLGHDDAYSDDK	0	0	0	0	0	0	0	0	0	0
225	Cry j 1	DDAYSDDKSMKVTVAF	298	796	3	14	16	71	30	4	5	5
233	Cry j 1	SMKVTVAFNQFGNCG	369	634	108	315	19	57	40	26	10	10
241	Cry j 1	NQFGNCGQRMPRARY	0	0	0	0	14	60	0	0	5	5
249	Cry j 1	QRMPRARYGLVHVANN	157	441	124	440	14	62	20	9	5	5
257	Cry j 1	GLVHVANNNDPWTIY	238	524	49	239	18	81	30	4	5	5
265	Cry j 1	NYDPWTIYAIGSSNP	64	202	0	0	0	0	10	0	0	0
273	Cry j 1	AIGSSNPITLSEGN	8	25	0	0	15	67	10	0	5	5
281	Cry j 1	TILSEGNSTAPNESY	722	963	22	106	24	107	60	4	5	5
289	Cry j 1	FTAPNESYKKQVTIRI	127	332	0	0	0	0	20	0	0	0
297	Cry j 1	KKQVTIRIGCKTSSC	27	58	0	0	34	134	20	0	10	10
305	Cry j 1	GCKTSSSCSNWVWQST	0	0	0	0	0	0	0	0	0	0
313	Cry j 1	SNWVWQSTQDVFNKA	579	746	0	0	35	115	60	0	10	10
321	Cry j 1	QDVFNKAYFVSSGKY	254	348	12	46	47	151	40	9	10	10
329	Cry j 1	YFVSSGKYEGGNIYTK	152	394	15	61	8	34	20	9	5	5
337	Cry j 1	EGGNIYTKKEAFNVEN	132	185	3	14	12	52	40	4	5	5
345	Cry j 1	KEAFNVENGNA TPQLT	140	253	4	20	0	0	40	4	0	0
353	Cry j 1	GNATPQLTKNAGVLTC	144	222	72	290	9	38	40	17	5	5
359	Cry j 1	LTKNAGVLTCSLSKRC	118	228	13	65	18	78	20	4	5	5
1	Cry j 2	MAMKLIAPMAFLAMQL	8	24	8	37	0	0	0	4	0	0
9	Cry j 2	MAFLAMQLIIMAAED	19	60	0	0	0	0	10	0	0	0
17	Cry j 2	IIMAAEDQSAQIMLD	9	28	0	0	0	0	0	0	0	0
25	Cry j 2	QSAQIMLDSWEKYLK	26	62	3	16	0	0	20	4	0	0

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)		
			LJ IgE+	SD	NLJ IgE+	St. Dev	LJ IgE-	SD	LJ IgE+	NLJ IgE+	LJ IgE-
33	Cry j 2	SVVEKYLRSNRSLRKV	0	0	7	25	0	0	0	9	0
41	Cry j 2	SNRSILRKVEHSRDAI	11	36	6	27	0	0	10	4	0
49	Cry j 2	EHSRDAINIFNVEKY	0	0	0	0	0	0	0	0	0
57	Cry j 2	NIFNVEKYGAVDGKH	28	61	9	29	0	0	20	9	0
65	Cry j 2	GAVDGKHDCTEAFST	0	0	3	12	0	0	0	4	0
73	Cry j 2	DCTEAFSTAWQAACKN	450	787	2	12	0	0	40	4	0
81	Cry j 2	AWQAACKNP SAMLVP	391	783	2	12	0	0	30	4	0
89	Cry j 2	PSAMLLVPGSKKFVN	340	946	30	102	0	0	30	13	0
97	Cry j 2	GSKKFWNNLFFNGPC	295	809	157	656	24	107	30	17	5
105	Cry j 2	NLFNGPCQPHFTFKV	20	62	89	413	0	0	10	9	0
113	Cry j 2	QPHFTFKVDGI IAAVQ	760	785	49	201	136	351	60	13	20
121	Cry j 2	DGI IAAVQNPASWKNN	888	840	114	358	226	549	70	22	25
129	Cry j 2	NPASWKNNRIWLQFAK	216	400	20	79	24	66	50	9	15
137	Cry j 2	RIWLQFAKLITGFTLMG	246	597	62	196	112	345	50	17	15
145	Cry j 2	LTGFTLMGKGVIDGQG	14	44	6	20	14	46	10	9	10
153	Cry j 2	KGVIDGQKQWWAGQC	29	91	0	0	0	0	10	0	0
161	Cry j 2	KQWWAGQCKWVNGREI	18	58	5	26	0	0	10	4	0
169	Cry j 2	KWVNGREICNDRDRPT	118	333	13	61	3	11	30	4	5
177	Cry j 2	CNDRDRPTAIKFDFST	41	88	18	71	20	62	20	9	10
185	Cry j 2	AIKFDFSTGLI IQGLK	187	442	15	63	140	416	40	9	25
193	Cry j 2	GLIIQGLKLMNSPEFH	47	69	7	35	9	42	40	4	5
201	Cry j 2	LMNSPEFHLVFGNCEG	77	160	5	26	14	47	30	4	10
209	Cry j 2	LVFGNCEGVKI IGISI	225	628	0	0	4	16	40	0	5
217	Cry j 2	VKIIGISITAPRDSFN	384	686	70	273	192	459	60	13	25
225	Cry j 2	TAPRDSPTDGDIFA	9	30	0	0	2	10	10	0	5
233	Cry j 2	TDGIDIFASKNFHLQK	33	78	9	46	16	70	20	4	5

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)			
			LJ IgE+	SD	NLJ IgE+	St. Dev	LJ IgE-	SD	LJ IgE+	NLJ IgE+	LJ IgE-	
241	Cry j 2	SKNFHLQKNTTIGTDD	176	329	0	0	9	40	30	0	5	
249	Cry j 2	NTTGTGDDCVAIGTGS	113	357	13	61	0	0	10	4	0	
257	Cry j 2	CVAITGTGSSNIVIEDL	146	461	12	59	5	22	10	4	5	
265	Cry j 2	SNIVIEDLICGPGHGI	166	468	3	13	4	17	30	4	5	
273	Cry j 2	ICGPGHGISIGSLGRE	0	0	5	18	16	53	0	9	10	
281	Cry j 2	SIGSLGRENRAEVSY	104	147	23	112	25	64	50	4	15	
289	Cry j 2	NSRAEVSYVHVNGAKF	363	548	0	0	33	108	50	0	15	
297	Cry j 2	VHVNGAKFIDTQNGLR	130	234	0	0	10	32	40	0	10	
305	Cry j 2	IDTQNGLRIKTWQGS	0	0	0	0	0	0	0	0	0	
313	Cry j 2	IKTWQGGSGMASHI IY	0	0	0	0	0	0	0	0	0	
321	Cry j 2	GMASHI IYENVENMINS	50	158	0	0	0	0	10	0	0	
329	Cry j 2	ENVENMINSNPILINQ	6	19	2	10	0	0	10	4	0	
337	Cry j 2	ENPILINQFYCTSASA	115	329	0	0	11	35	20	0	10	
345	Cry j 2	FYCTSASACQNQRSAV	26	81	0	0	9	38	10	0	5	
353	Cry j 2	CQNQRSAVQIQDVTYK	86	237	0	0	8	34	20	0	5	
361	Cry j 2	QIQDVTYKNIRGTSAT	474	693	2	10	95	306	50	4	15	
369	Cry j 2	NIRGTSATAAAIQLKC	298	650	7	24	24	78	30	9	10	
377	Cry j 2	AAAIQLKCSDSMPCCKD	70	148	0	0	15	48	30	0	10	
385	Cry j 2	SDSMPCCKDIKLSDISL	19	61	0	0	0	0	10	0	0	
393	Cry j 2	IKLSDISLKLTSKGKIA	602	848	7	26	17	56	60	9	10	
401	Cry j 2	KLTSKGKIASCLNDNAN	809	832	21	103	28	78	70	4	15	
409	Cry j 2	SCLNDNANGYFSGHVI	49	156	0	0	0	0	10	0	0	
417	Cry j 2	GYFSGHVIPACKNLSP	0	0	0	0	9	39	0	0	5	
425	Cry j 2	PACKNLSPSAKRKESK	99	262	0	0	0	0	20	0	0	
433	Cry j 2	SAKRKESKSHKPKTV	0	0	0	0	0	0	0	0	0	
441	Cry j 2	SHKHPKTVMVENMRAY	0	0	2	11	0	0	0	4	0	

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)					
			LIJ IgE+	SD	NLJ IgE+	St. Dev	LIJ IgE-	SD	LIJ IgE+	NLJ IgE+	LIJ IgE-	SD	NLJ IgE+	LIJ IgE-
449	Cry j 2	MVENMRAYDKGNRTRI	0	0	4	18	0	0	0	0	4	0	4	0
457	Cry j 2	DKGNRTRILLGSRPPN	24	76	14	71	0	0	0	10	4	0	4	0
465	Cry j 2	LLGSRPPNCTNKCHGC	0	0	0	0	0	0	0	0	0	0	0	0
473	Cry j 2	CTNKCHGCSGPCAKLV	0	0	0	0	0	0	0	0	0	0	0	0
481	Cry j 2	SPCKAKLVIVHRIMPQ	0	0	5	27	0	0	0	0	4	0	4	0
489	Cry j 2	IVHRIMPQEYYPQRWI	0	0	0	0	0	0	0	0	0	0	0	0
497	Cry j 2	EYYPQRWICSCHGKIY	7	21	13	65	0	0	10	4	0	0	4	0
499	Cry j 2	YPQRWICSCHGKIYHP	63	199	14	47	0	0	10	9	0	0	9	0
1	Cry j IFR	MGGSRVLIIGGTGYIG	0	0	4	19	0	0	0	4	0	0	4	0
9	Cry j IFR	IGGTGYIGRHVTNASL	0	0	0	0	0	0	0	0	0	0	0	0
17	Cry j IFR	RHVTNASLAQGHPTFL	0	0	0	0	0	0	0	0	0	0	0	0
25	Cry j IFR	AQGHPTFLLVREITPS	0	0	0	0	0	0	0	0	0	0	0	0
33	Cry j IFR	LVREITPSNPEKAQLL	0	0	0	0	0	0	0	0	0	0	0	0
41	Cry j IFR	NPEKAQLLESFTSKGA	0	0	0	0	0	0	0	0	0	0	0	0
49	Cry j IFR	ESFTSKGATLVQGSID	0	0	0	0	0	0	0	0	0	0	0	0
57	Cry j IFR	TLVQGSIDDHASLVAA	0	0	0	0	0	0	0	0	0	0	0	0
65	Cry j IFR	DHASLVAAALKKVDVVI	0	0	0	0	0	0	0	0	0	0	0	0
73	Cry j IFR	LKKVDWISTLGAPQI	0	0	0	0	0	0	0	0	0	0	0	0
81	Cry j IFR	STLGAPQIADQFNLIK	0	0	0	0	0	0	0	0	0	0	0	0
89	Cry j IFR	ADQFNLIKAKEVGTI	0	0	14	49	0	0	0	9	0	0	9	0
97	Cry j IFR	AIKEVGTIKRFFPSEF	0	0	18	61	0	0	0	9	0	0	9	0
105	Cry j IFR	KRFFPSEFGNDVDKHH	0	0	10	36	0	0	0	9	0	0	9	0
113	Cry j IFR	GNDVDKHHAVEPMKSM	0	0	22	67	0	0	0	13	0	0	13	0
121	Cry j IFR	AVEPMKSMFDLKIILR	0	0	26	66	0	0	0	17	0	0	17	0
129	Cry j IFR	FDLKIILRRTIEAEGI	0	0	11	53	0	0	0	4	0	0	4	0
137	Cry j IFR	RTIEAEGIPHTYWP	0	0	0	0	0	0	0	0	0	0	0	0

Peptide start	Antigen	Sequence	Average response magnitude (SFC)						Average response frequency (%)			
			LIJ IgE+	SD	NLJ IgE+	St. Dev	LIJ IgE-	SD	LIJ IgE+	NLJ IgE+	LIJ IgE-	IgE-
145	C γ 1 IFR	PHTYVVPHCFAGYFLT	0	0	14	52	0	0	0	0	9	0
153	C γ 1 IFR	CFAGYFLTNLAQGLA	0	0	8	38	0	0	0	0	4	0
161	C γ 1 IFR	NLAQLGLAAPPRDKIV	0	0	3	15	0	0	0	0	4	0
169	C γ 1 IFR	APPRDKIVYIGDGTK	0	0	0	0	3	13	0	0	0	5
177	C γ 1 IFR	IYGDGTTKAVYMKED	0	0	0	0	0	0	0	0	4	0
185	C γ 1 IFR	AVYNKEEDI GTFTIKA	0	0	3	12	0	0	0	0	4	0
193	C γ 1 IFR	IGTFTIKAVDDPRTLN	0	0	3	14	0	0	0	0	4	0
201	C γ 1 IFR	VDDPRTLNKTLYLKPP	0	0	4	19	0	0	0	0	4	0
209	C γ 1 IFR	KTLYLKPPANTISTND	0	0	0	0	0	0	0	0	0	0
217	C γ 1 IFR	ANTIISTNDLVALWEAK	0	0	0	0	0	0	0	0	0	0
225	C γ 1 IFR	LVALWEAKIGKLEKV	0	0	0	0	0	0	0	0	0	0
233	C γ 1 IFR	IGKLEKVYLSEEQVL	0	0	0	0	0	0	0	0	0	0
241	C γ 1 IFR	YLSEEQVLKLLQDTPF	0	0	0	0	0	0	0	0	0	0
249	C γ 1 IFR	KLLQDTPFPFGTFMYSI	0	0	0	0	0	0	0	0	0	0
257	C γ 1 IFR	PGTFMVSIFHTIYVKG	0	0	0	0	5	20	0	0	0	5
265	C γ 1 IFR	FHTIYVKGDTNFQIG	0	0	0	0	0	0	0	0	0	0
273	C γ 1 IFR	DQTNFQIGPDGVEASA	0	0	0	0	0	0	0	0	0	0
281	C γ 1 IFR	PDGVEASALYPDVKYT	0	0	0	0	0	0	0	0	0	0
289	C γ 1 IFR	LYPDVKYTTVEEYISA	0	0	8	39	26	74	0	4	15	15
291	C γ 1 IFR	PDVKYTTVEEYISAFV	0	0	0	0	8	34	0	0	0	5

SD- standard deviation

Table 3

Correlation of T cell responses between cohorts for Cry j 1, Cry j 2 and Cry j IFR was performed by Spearman's rank correlation analysis. $P < 0.05$ is considered significant.

Cry j 1 R ² values		Cohort			Cry j 1 p values			Cohort		
		LJ E+	NLJ E+	LJ IgE-		LJ E+	NLJ E+	LJ IgE-		
Cohort	LJ E+		0.34	0.19		LJ E+		<0.001	0.003	
	NLJ E+	0.34		0.09		NLJ E+	<0.001		0.043	
	LJ IgE-	0.19	0.09			LJ IgE-	0.003	0.043		
Cry j 2 R ² values		Cohort			Cry j 2 p values			Cohort		
		LJ E+	NLJ E+	LJ IgE-		LJ E+	NLJ E+	LJ IgE-		
Cohort	LJ E+		0.16	0.4		LJ E+		0.001	<0.001	
	NLJ E+	0.16		0.14		NLJ E+	0.001		0.002	
	LJ IgE-	0.4	0.14			LJ IgE-	<0.001	0.002		
Cry j IFR R ² values		Cohort			Cry j IFR p values			Cohort		
		LJ E+	NLJ E+	LJ IgE-		LJ E+	NLJ E+	LJ IgE-		
Cohort	LJ E+		n.a.	n.a.		LJ E+		n.a.	n.a.	
	NLJ E+	n.a.		0.03		NLJ E+	n.a.		0.293	
	LJ IgE-	n.a.	0.03			LJ IgE-	n.a.	0.293		

Table 4

Inferred HLA allele restriction analysis performed using the RATE analysis tool. A- Allele, R- Responder, RF- relative frequency, OR-odds ratio. P<0.05 is considered significant.

Antigen	Start	Peptide	Allele(s) of inferred restriction	Number of donors				Percentage of donors				No. of donors	RF	OR	P-value
				A+R+	A- R+	A+R-	A- R-	A+R+	A- R+	A+R-	A- R-				
Cry j 1	233	SMKVTVAFNQFGPNCG	DPB1*05:01,DRB5*01:01,DRB1*04:01	9	0	7	16	28%	0%	22%	50%	32	2.0	inf	0.001
Cry j 2	121	DGIIAAYQNPAWKNN	DRB1*15:02,DRB5*01:01,DRB5*01:02,DRB5*02:02	8	2	3	19	25%	6%	9%	59%	32	2.3	25.3	0.001
Cry j 2	113	QPHFTTFKVDGIIAAYQ	DPB1*02:01,DPB1*09:01,DRB1*15:02,DPB1*01:01	7	0	8	17	22%	0%	25%	53%	32	2.1	inf	0.002
Cry j 2	393	IKLSDISLKLTSKGIA	DPB1*02:01,DRB1*15:02,DRB5*01:02	6	0	6	20	19%	0%	19%	63%	32	2.7	inf	0.001
Cry j 2	217	VKIIIGISITAPRDSFN	DRB1*15:02,DRB1*04:03,DRB5*01:01,DRB5*01:02,DRB1*14:01	6	1	5	20	19%	3%	16%	63%	32	2.5	24.0	0.003
Cry j 2	401	KLTSKGIIASCLNDNAN	DRB1*04:04,DQB1*03:03,DRB1*15:02,DRB1*04:01	5	1	4	22	16%	3%	13%	69%	32	3.0	27.5	0.003
Cry j 2	129	NPASWKNNRILWLQFAK	DRB1*15:01,DPB1*18:01,DPB1*11:01,DRB1*15:02	5	1	4	22	16%	3%	13%	69%	32	3.0	27.5	0.003
Cry j 2	89	PSAMLLVPGSKKFFVN	DRB1*13:03,DRB1*15:02,DRB1*03:02,DRB5*01:02	5	1	1	25	16%	3%	3%	78%	32	4.4	125.0	0.000
Cry j 1	321	QDVFYNGAYFVSSGKY	DPB1*18:01,DPB1*11:01,DPB1*05:01,DRB3*01:01	5	0	6	21	16%	0%	19%	66%	32	2.9	inf	0.002
Cry j 2	137	RIWLQFAKLITGFTLMG	DPB1*18:01,DPB1*11:01,DRB1*15:02,DRB5*01:02,DPB1*09:01,DPB1*03:01	5	2	2	23	16%	6%	6%	72%	32	3.3	28.8	0.002
Cry j 1	281	TILSEGNSTAPNESY	DRB1*04:05,DRB1*15:02,DRB1*04:01	5	1	4	22	16%	3%	13%	69%	32	3.0	27.5	0.003
Cry j 1	129	VFIKRVSNVI IHGLYL	DRB1*14:04,DRB1*04:04,DRB1*15:02,DRB5*01:02	5	3	1	23	16%	9%	3%	72%	32	3.3	38.3	0.002
Cry j IFR	121	AVEPMKSMFIDLKIKLR	DRB1*16:02,DRB5*02:02,DRB1*13:01,DRB1*03:01	4	0	2	26	13%	0%	6%	81%	32	5.3	inf	0.000
Cry j 2	81	AWQAACKNPASMLLVP	DRB1*15:02,DRB1*13:03,DRB1*03:01	4	0	5	23	13%	0%	16%	72%	32	3.6	inf	0.004
Cry j 1	257	GLVHVANNYDPWTIY	DRB1*04:05,DRB1*15:02,DQB1*05:03	4	0	5	23	13%	0%	16%	72%	32	3.6	inf	0.004
Cry j 1	353	GNATPQLTKNAGVLTG	DRB3*03:01,DRB1*15:02,DRB1*13:03,DRB1*13:02	4	4	2	22	13%	13%	6%	69%	32	2.7	11.0	0.023
Cry j 1	89	LWIIIFSGNMNIIKLKMP	DPB1*11:01,DRB1*15:02,DPB1*09:01,DRB1*04:03,DRB5*01:02,DRB1*14:01	4	2	1	25	13%	6%	3%	78%	32	4.3	50.0	0.002
Cry j 2	361	QIQDVTYKNIRGTSAT	DRB1*15:01,DRB1*15:02,DRB5*01:02,DRB1*04:01	4	0	6	22	13%	0%	19%	69%	32	3.2	inf	0.006
Cry j 1	209	SNNLFTNHHKVMLLGH	DRB1*08:01,DRB1*04:01,DRB1*03:01	4	1	3	24	13%	3%	9%	75%	32	3.7	32.0	0.004
Cry j 1	313	SNWVWQSTQDVFYNGA	DRB1*09:01,DRB1*15:02,DRB1*04:01,DPB1*09:01	4	0	5	23	13%	0%	16%	72%	32	3.6	inf	0.004